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BOX PROVISIONAL
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No: UOFW115861

PROVISIONAL APPLICATION COVER SHEET

Seattle, Washington 98101

August 1, 2000

TO THE ASSISTANT COMMISSIONER FOR PATENTS:

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(c).

Transmitted herewith for filing by EXPRESS MAIL is the complete provisional patent application of inventors:

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Title of Invention: A STRATEGY TO ENHANCE THE HEALING OF BIOMATERIALS

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ENCLOSED ARE:

__X__ 1. A provisional application for patent consisting of 13 pages of specification and drawings.

__X__ 2. Our Check No. 119150 in the amount of $150.00 to cover the total provisional filing fee.

__X__ 3. The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. The names of the U.S. Government agencies and the Government contract numbers are: Government Agency: National Science Foundation; Government Contract No: EEC-9529161; and Government Agency: National Institutes of Health; Government Contract No: AR45418.

__X__ 4. A filing date in accordance with 37 C.F.R. § 1.10 is requested. The Express Mail Certificate appears below.

Respectfully submitted,

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EXPRESS MAIL CERTIFICATE

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A Strategy to Enhance the Healing of Biomaterials

This invention was developed under NSF Grant EEC-9529161, "University of Washington Engineered Biomaterials", and NIH Grant AR45418, "The Extracellular Matrix in Mice Lacking Thrombospondin 2"

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Summary of the Invention

This technology describes a bioengineered system that enhances healing of wounds, tissue remodeling, and the biocompatibility of medical materials. This system can be designed to utilize both sense and antisense DNA technology and immobilized biomolecules to trigger these beneficial tissue responses. These approaches include the delivery of anti-sense DNA or anti-sense oligos for the gene encoding thrombospondin 2 (TSP 2), the delivery of sense DNA for osteopontin (OPN), the presentation of chemically immobilized OPN/ OPN-derived peptides, or the delivery and/or presentation of TSP 2 antibodies or blocking peptides at the surface of the material. In general, any molecule that can interfere with TSP 2 synthesis or function, or any molecule that can introduce OPN or functional OPN fragments is included in this invention. To deliver these molecules a device in the form of a polymer or film can be used directly on skin wounds, or as a coating for biomaterials used in implantable medical devices. The device is designed to deliver an effective amount of biological signals to the surrounding tissue that are capable of promoting wound healing and minimizing scarring through modulation of matrix remodeling and neovascularization and by controlling the activity of inflammatory cells.

Background

In virtually all cases, there results from the contact of blood and tissues with synthetic materials a negative reaction or rejection of the material termed the "foreign body response". The basis for this reaction is complex. However, the failure of the material to react specifically with the body's proteins and the activities of various cellular components of the inflammatory and immunological systems results in the incompatibility of the materials. The material becomes encapsulated by a fibrous sheath, or, as with skin wounds, causing the injury to enter a chronic state in which healing is delayed, or subject to intense scarring as with serious burn injury. For even the smallest of implantable medical devices, the healing of the implant wound site is complicated by the foreign body reaction.

Critical to the healing of wounds is the need to supply the new, growing tissue with oxygen. Typically, chronic wounds and medical implants suffer from an inability of the body to deliver a sufficient blood supply to the area. This deficiency can be overcome by enhancing the creation of new blood vessels through the processes of angiogenesis and vasculogenesis.

Past efforts to enhance wound healing have focused on therapeutic application of hydrogel polymers containing growth factors, cytokines, and bioactive peptides. A common limitation for most of these therapies has been the failure to formulate the agent and deliver an effective dose to appropriate and precise target sites in the healing tissue. The therapeutic protein agents are labile and easily degraded in the milieu of proteolytic enzymes at the wound site.

A variety of studies performed in vitro and in vivo, including cell cultures and wild-type and knockout animals, have shown that the matricellular protein thrombospondin 2 (TSP 2) plays a direct role in the modulation of matrix remodeling and neovascularization of wounded tissue. In particular, mice which lack the gene for TSP 2 (TSP2-null) exhibit an altered foreign body response associated with loose capsule organization and enhanced vascularization of wounds and implant sites in tissues. Thus, the enhanced vascularization observed through the ablation of

TSP 2 expression is explained. In addition, TSP2-null mice display altered matrix organization at the wound site which is linked to reduced scarring.

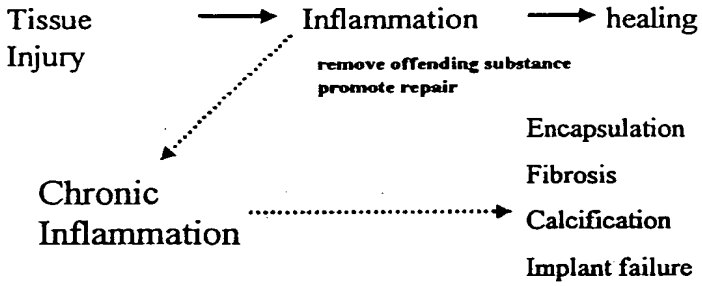
Also relevant is the activity of macrophages in the wound or implant site. When confronted with a foreign material, macrophages have been shown to enter into an inflammatory state that is associated with release of specific cytokines and the transition to a state of frustrated phagocytosis that leads to a multinucleate foreign body giant cell (FBGC) phenotype through membrane fusion events and syncytia formation. It has been shown that osteopontin (OPN) plays a key role in controlling these processes, and specifically effective is the immobilization of the protein at the material surface. Immobilized OPN is shown here to promote a more appropriate "healing-state" of macrophages surrounding the implanted material, the subsequent reduction of the foreign body encapsulation and appearance of FBGC's.

Inflammation can be thought of as the key process by which offending substances (foreign materials and infectious organisms) can be removed from a wound site. It is well established that this aspect of the inflammatory response is essential for wound healing, particularly the role of macrophages. The macrophage signals other cells, especially fibroblasts, to migrate to the wound area and remodel the extracellular matrix. The macrophage is also responsible for destroying infectious agents and presenting antigens to the cellular immune system and for "cleaning" up the wound site by acquiring a phagocytic phenotype.

It is essential for normal wound healing that the inflammatory stage subsides. Advertent establishment of "permanently" activated macrophages leads to a state of chronic inflammation and prevents the normal subsequent remodeling and healing of the wound. An implanted biomaterial resides in the midst of this wound healing process, and is often associated with a state of chronic inflammation- directed by macrophages that have not subsided in these activities. These concepts and resulting consequences are depicted in Figure 1.

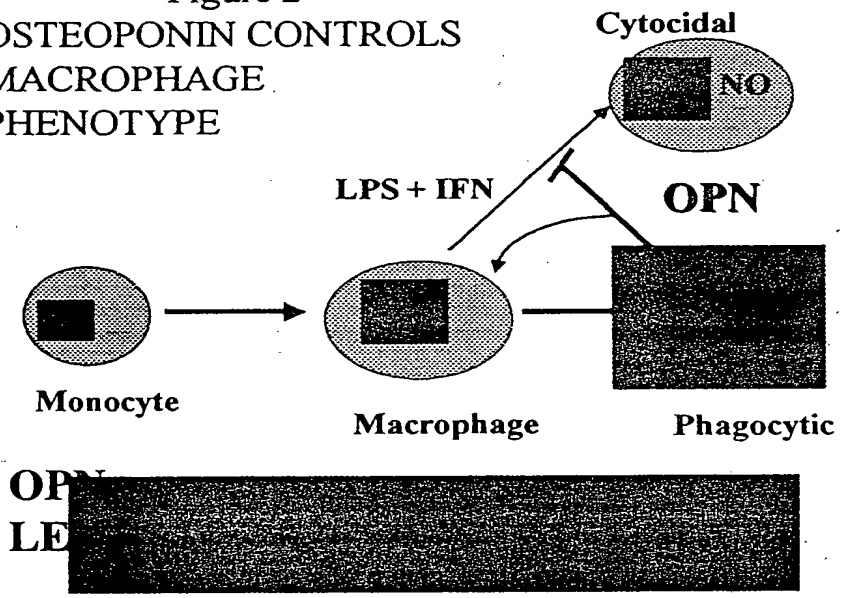
Figure 1
INFLAMMATION

Fundamentally a protective mechanism that attempts to promote wound healing



A large body of evidence supports the role of osteopontin in the regulation of macrophage phenotype. As depicted in Figure 2, osteopontin was investigated for its ability to ablate the chronic inflammatory macrophage phenotype, particularly with respect to implanted biomaterials.

Figure 2
OSTEOPONIN CONTROLS MACROPHAGE PHENOTYPE



Taken together, the knowledge of the biology of TSP 2 and OPN provides the opportunity for a biomaterial to be designed to heal in the tissue. By blocking the activity of TSP 2 and promoting the activity of OPN (separately or together in a single material), both the inhibition of the foreign body response and the promotion of neovascularization can be achieved.

Invention

A method involving the localized delivery of plasmid DNA incorporated into a collagenous matrix was utilized. In brief, millipore filters coated with a plasmid-collagen mixture (GAM: gene-activated matrix) were implanted in TSP2-null and control mice for a 2-4 week period. Plasmids encoding TSP2 sense and anti-sense cDNA, as well plasmids encoding green fluorescence protein GFP as a reporter gene, were employed.

The data (Figure 3) indicate that gene delivery via the GAM could effectively influence the extent of capsule neovascularization. Thus, TSP2-null animals implanted with a TSP2-sense GAM-coated filter display a decrease in blood vessel density (compare columns 7 and 4) and wild-type animals implanted with an anti-sense TSP2 GAM-coated filter display an increase in blood vessel density (compare columns 6 and 2). No change is observed in wild-type animals implanted with a TSP2 sense GAM-coated filter (compare column 5 and 2). Coating of filters

with matrix alone resulted in a reduction in the number of blood vessels in both wild type (compare columns 8 and 2) and TSP2-null mice (compare columns 9 and 4).

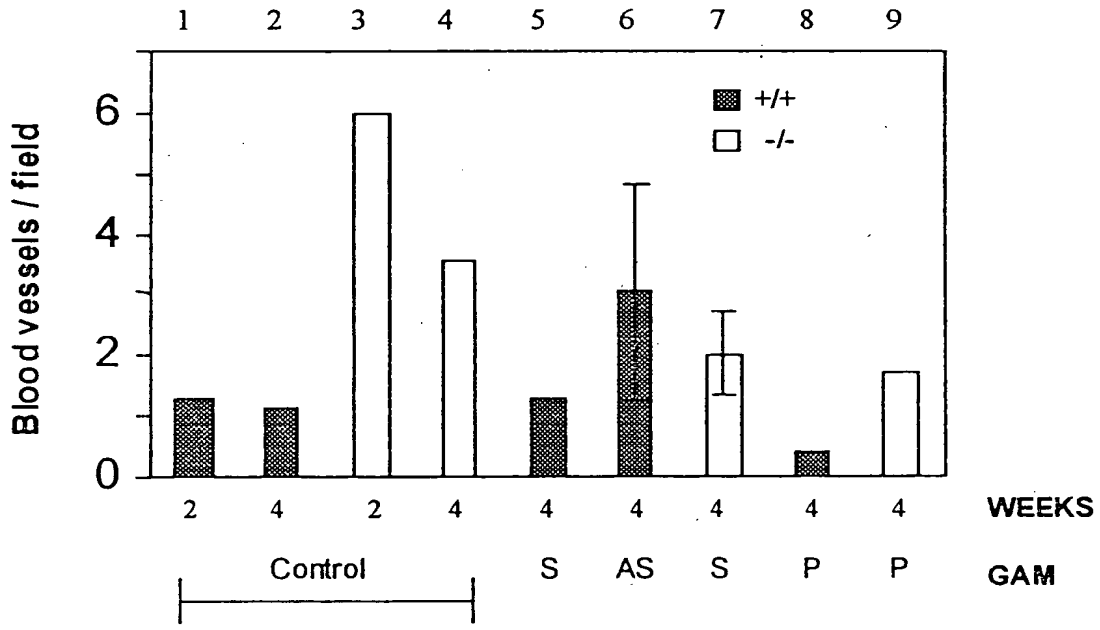


Figure 3. GAM-induced alteration of foreign body capsule neovascularization. Bars represent the number of blood vessels per high-powered field in capsules surrounding implanted millipore filters. Implantation periods in number of weeks are shown. Control indicates the lack of implant coating (no GAM). S and AS indicate the presence of GAM containing plasmids with sense and anti-sense TSP2 cDNA respectively. P indicates the presence of collagen matrix alone (no plasmid).

The ability of a TSP2 anti-sense cDNA-encoding GAM to increase the neovascularization of foreign body capsules in wild type animals suggests that this strategy could be effective in the beneficial elimination of the anti-angiogenic activity of TSP2. It is significant to note that the delivery of anti-sense TSP2 was effective, despite the overall reduction in vascularity caused by the presence of the collagen matrix alone. This anti-sense cDNA based-

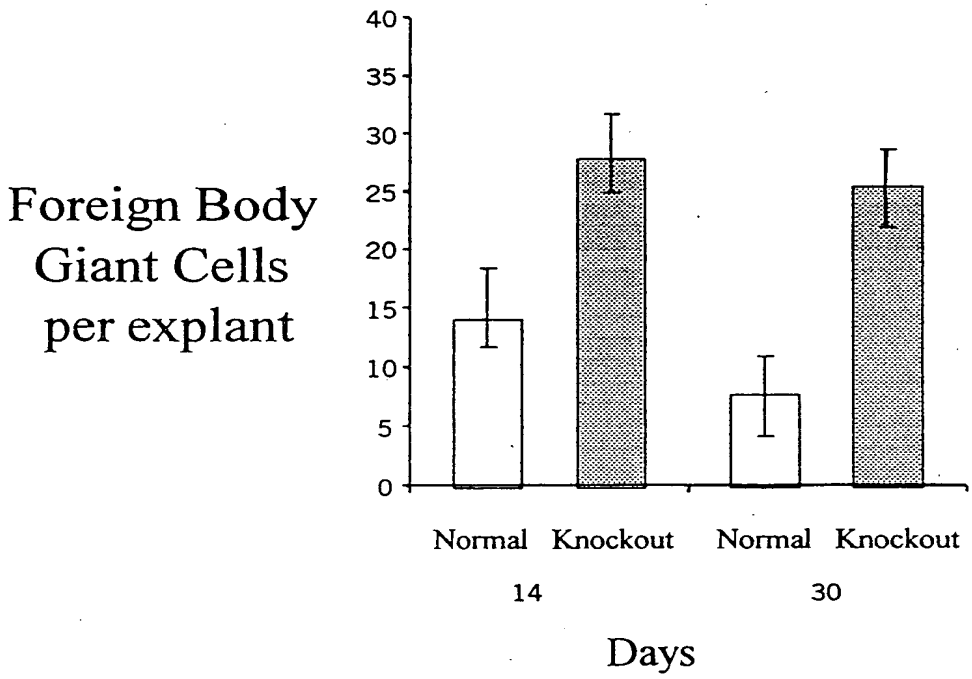
strategy can be applied locally during tissue remodeling when enhancement of the angiogenic response and alteration of matrix remodeling are desired, such as in wound healing, the response to a biomaterial implant, or any other application that can benefit from enhanced neovacsularization.

One of the hallmarks of the foreign body response is the appearance of foreign body giant cells or macrophages that have fused together as a result of encountering an implanted foreign material. As many as 100 cells fuse to form a syncytium containing as many as one hundred nuclei. It is thought that these giant cells are the result of the macrophage's inability to phagocytize the large material present in the wound. To address the role of osteopontin in this pathological reaction, we utilized genetic knockout mice, which fail to express osteopontin.

As subject biomaterials, fixed bovine pericardium samples were implanted into a series of mice and analyzed at various times for the appearance of foreign body giant cells. As shown in Figure 4, the osteopontin null mice (OPN^-/ OPN^-) characteristically have high levels of giant cells surrounding the implant, compared to control mice (OPN^+/ OPN^+) which have normal expression of osteopontin. Of further significance, note that the levels of foreign body giant cells in the normal mice begin to subside with time, perhaps reflecting the transient nature of osteopontin's role.

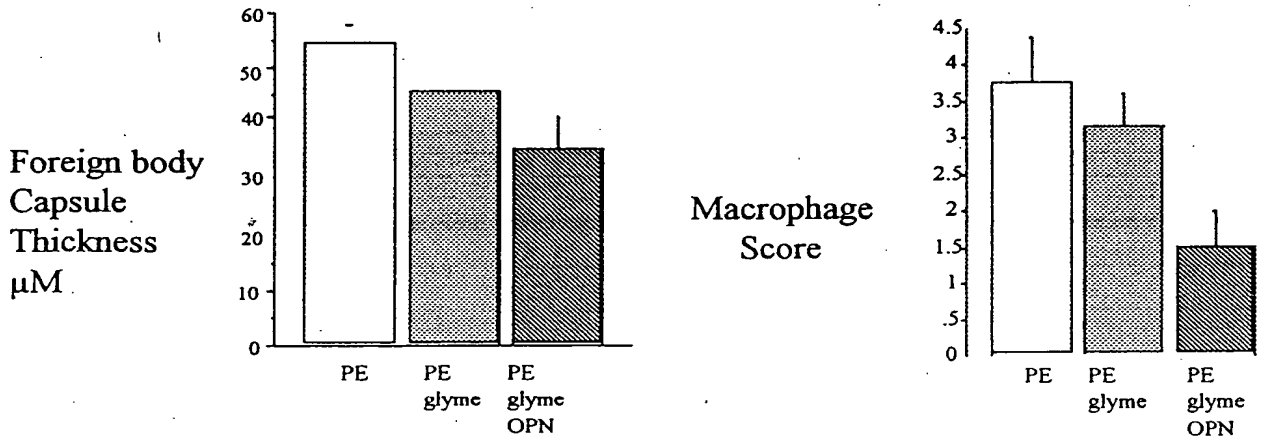
Figure 4

Osteopontin Reduces Foreign Body Giant Cells



In another experiment (Figure 5), tissue sections were quantified for foreign body capsule thickness and macrophages following 4 weeks of implantation of polyethylene disks S.C. in mice. Uncoated disks were compared with a non-fouling (RFGD tetraglyme) coating and also with osteopontin covalently immobilized to the glyme coating. From the data shown in Figure 4, immobilized osteopontin (OPN) is associated with a marked reduction in fibrous capsule thickness and macrophage infiltration. Thus, it is possible to utilize osteopontin in this way to divert the chronic inflammatory reaction to implanted biomaterials and direct the tissue toward a favorable healing outcome.

Figure 5
Osteopontin immobilization on stealth surfaces promotes implant healing



Broadening

The anti-TSP 2 DNA delivery system (or any other approach that utilizes TSP2 as a molecular target) can be applied to a wide variety of medical products, including but not limited to: hydrogels for direct therapy of wounds and burns; percutaneous access devices such as in-dwelling catheters, optic and electrical connectors, biosensors or left-ventricular devices; vascular prostheses such as grafts or mechanical hear valves; orthopedic prosthesis such as hip or knee joints; tissue engineering scaffolds such as are used in artificial skin devices; implantable biosensors and membranes.

The delivery of DNA, or other biomolecules, can also be accomplished by different methods of coating biomaterials and preparing standard materials including, but limited to: polyurethanes, polycarbonates, polyethylenes, other plastics, metals, porous biomaterials, degradable polymers such as poly (lactic acid) glycols or poly (galactic acid) ethers. Also pertinent are delivery systems based upon controllable design parameters such as pH, external energy, temperature and mechanical forces.

In addition to the scheme presented in the preferred mode of the invention to immobilize OPB covalently to bioprosthetic tissue and polyurethanes, a variety of chemical means can be employed. Virtually any material, when appropriately modified to include functional groups (carboxyl, amide, amino, ether, hydroxyl, cyano, nitrido, sulfanamido, acetylnic, ethynic or epoxide, silanic, anhydric, succinimic, azido) at its surface can be used for protein immobilization. Coupling chemistries include but are not limited to the formation of esters, ethers, amides, azido and sulfanamido derivatives, cyanate and other linkages to the functional groups available on proteins.

Of specific importance is a form of this invention in which the biology of OPN and TSP 2 are captured onto a single biomaterial. Such a biomaterial would, in a preferred mode, deliver a TSP2 synthesis or function inhibitor and also present immobilized OPN to the surrounding tissue. This can be accomplished in a variety of constructs including but not limited to multicomponent designs, laminates, biphasic constructs, and or surfaces patterned to achieve simultaneously the DNA delivery and cellular signally required. An example of the latter type of construct is diagrammed below in Figure 6. Figures 7 and 8 depict alternative constructs designed to block the expression or activity of TSP2 and enhance the expression or capture the activity of OPN.

Figure 6
Material designed to release anti-sense TSP-2 DNA and
Containing patterns of immobilized OPN

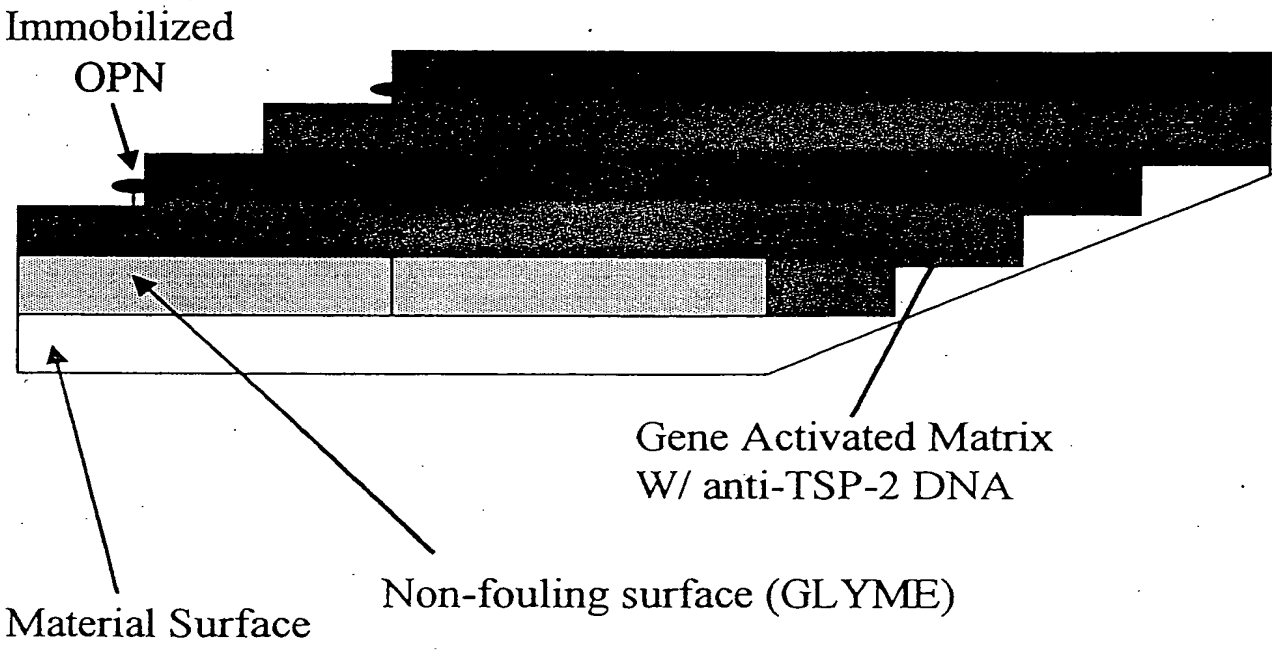


Figure 7
Material designed to release anti-sense TSP-2 DNA and Sense OPN DNA

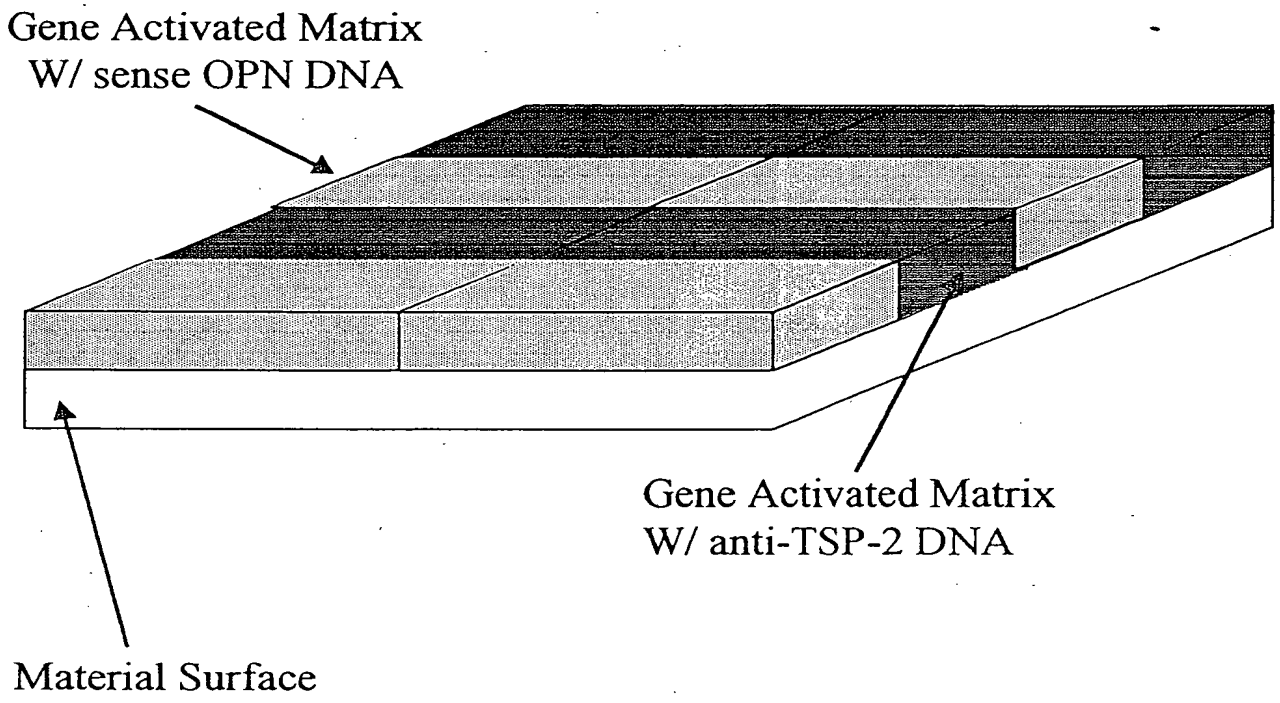
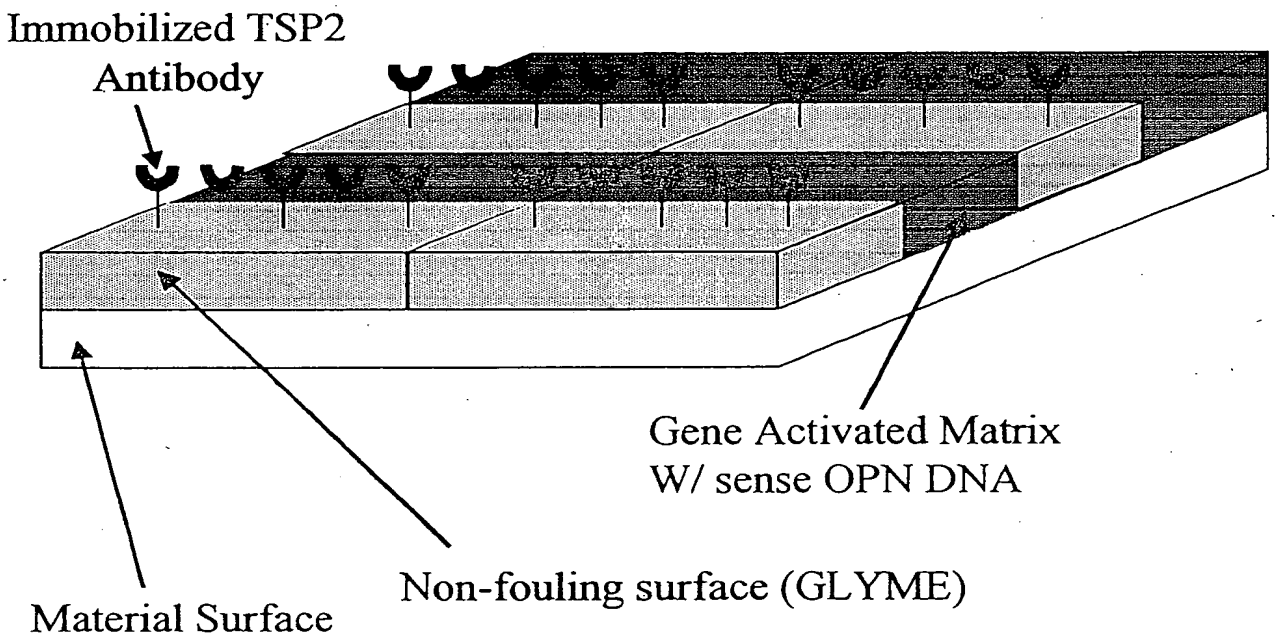


Figure 8
Material designed to release sense OPN DNA and
Containing patterns of immobilized TSP2 antibody



List of Figures

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Applicant(s): Bornstein et al.
Title: A STRATEGY TO ENHANCE THE HEALING OF BIOMATERIALS

The following have been received in the U.S. Patent and Trademark Office on the date stamped hereon via Express Mail No. EM589589876US:

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